REMARKS

Claims 1-35 are pending. Claims 2, 4, 8, 20, 22 and 26 have been cancelled without prejudice or disclaimer.

As a preliminary matter, the legend to Figure 1, and claims 1 and 19 were amended to recite cell handling module, rather than sample handling well. This was done in the interest of clarity and to be consistent with the "Detailed Description of the Invention" which discusses cell handling modules, rather than sample or cell handling wells. See for example, page 10, line 9, through page 14, line 23. Similarly, claims 5, 6, 7, 9, 23, 24, 25, and 26 were amended to recite module rather than well.

Objection to the Drawings under 37 C.F.R. § 1.83(a):

The drawings are objected to under 37 C.F.R. § 1.83(a) because they do not show every feature specified in the claims. Applicants have provided Figure 2, which shows the features specified in the claims. Specifically, preferred embodiments for the cell handling module, and the reaction module are shown, including: (1) the cell lysis module; (2) the cell removal module; (3) the cell concentration module; (4) the cell separation module; (5) the electrophoresis module; (6) the nucleic acid amplification module; (7) the thermal module; (8) the pumps; and, (9) the valves. Applicants note that the cell handling module, component **40** and the reaction module, component **45** were shown in Figures 1B-1D.

Applicants submit that the preferred embodiments shown in Figure 2 do not constitute new matter. A cell handling module (i.e. sample handling well) **40** is depicted in Figure 1. Preferred embodiments of the cell handling module are discussed in the specification beginning at page 10, line 9, through page 14, line 23. Preferred embodiments of the reaction module **45**, such as the nucleic acid amplification module and the thermal module are discussed in the specification beginning at page 15, line 24, through page 16, line 30, and page 38, lines 16-23.

"On Chip" pumps, that is pumps located within the microchannels **15** and **20** of the microfluidic devices are discussed in the specification at page 39, lines 5-32. The specification also discusses the types of pumps which may be used in the devices of the invention, including electroosmotic and electrohydrodynamic pumps (see page 39, lines 18-

32).

The specification discusses valves at page 40, lines 28-33. The valves may be located in the microchannels either preceding or following a module (see page 40, lines 28-29); an embodiment is shown in Figure 2H.

As the preferred embodiments illustrated in Figure 2 show the feature specified in the claims and do not constitute new matter, applicants respectfully request withdrawal of the objection under 37 C.F.R. § 1.83(a).

Rejection under 35 U.S.C. § 112, first paragraph:

Claims 1-35 are rejected under 35 U.S.C. § 112, first paragraph. Specifically, the Examiner states that the specification is devoid of any reference to the drawings. Applicants have amended the relevant paragraphs in the specification to reference the structures recited in the claims. Thus, the paragraph beginning at line 9, page 10 has been amended such that the modules listed are referenced to the structures illustrated in Figure 2. Specific reference to cell lysis module recited in claims 5 and 23 appears in the paragraph beginning at line 8 of page 11. Specific reference to the cell removal module recited in claims 6 and 24 appears in the paragraph beginning at line 8 of page 12. Specific reference to the cell concentration module recited in claims 7 and 23 appears in the paragraph beginning at line 12 of page 12. Specific reference to the cell separation module recited in claims 9 and 27 appears in the paragraph beginning at line 26 of page 11. Specific reference to the electrophoresis module recited in claims 10 and 28 appears in the paragraph beginning at line 24 of page 14. Specific reference to the nucleic acid amplification module recited in claims 11 and 29 appears in the paragraph beginning at line 24 of page 16. Specific reference to the thermal module recited in claims 13 and 31 appears in the paragraph beginning at line 16 of page 38.

Applicants note that claims 4, 8, 22 and 26 have been cancelled and so the rejection is moot.

The reaction module recited in claims 11 and 29 is depicted in Figure 1D. In addition, the paragraph beginning at line 24 of page 15 has been amended to provide specific reference to the reaction module.

In accordance with the description contained in the specification beginning at line 5 of page 39, Figure 2H depicts one location for this element in the microfluidic device of the invention. In addition, specific reference to the pump has been provided in the paragraph beginning at line 5 of page 39. Likewise, Figure 2H depicts one location for the valves of the invention. Specific reference to the valves has been provided in the paragraph beginning at line 28 of page 40.

Finally, Applicants note that M.P.E.P. § 608.01(p) allows material to be incorporated by reference. Incorporation of "essential material" may be done by reference to issued U.S. patents and incorporation of "non essential material" may be done by reference to patents or applications published in the U.S. or foreign countries and in other non-patent publications. Applicants note that the material incorporated by reference used to describe the various structural components of the invention are issued U.S. patents. See for example, page 11, line 10 reciting an issued U.S. patent for the cell lysis module; page 14, line 25 reciting issued U.S. patents for the electrophoresis module; page 38, line 19 reciting issued U.S. patents for the thermal module; and, page 39, lines 25-26 and 31, reciting issued U.S. patents for the pumps.

Accordingly, applicants respectfully submit that the specification provides a written description of the invention and request withdrawal of the invention.

Rejection under 35 U.S.C. § 112, second paragraph:

Claims 1-35 are rejected under 35 U.S.C. § 112, second paragraph for being indefinite.

The Examiner states that claims 1, 2, and 19, 20 are in conflict with each other. Claims 2and 20 have been cancelled, thus the rejection is moot.

The Examiner states the recitation of " said sample handling well is a reagent storage well" conflicts with the description of the module. Claim 3 has been amended to clarify the relationship of the storage well and thus the rejection is moot.

Claims 4 and 22 have been cancelled. Claims 9 and 27 have been amended to be consistent with the specification beginning at line 26, page 11. Thus, the rejection is moot.

Claims 11 and 29 have been amended to clarify that the reaction module is separate from the cell handling module. Thus, the rejection is moot.

Claims 13 and 31 have been amended to clarify that the thermal module is an embodiment of the reaction module. Thus, the rejection is moot.

Claims 5, 6, 7, 23, 24 and 25 have been amended to recite module. Support for these amendments is found in the specification as outlined above. Claims 8 and 26 have been cancelled. Thus, the rejection is moot.

The Examiner states that claims 14, 17, 32 and 34 are indefinite because no structural relationship is recited between the pump and the valve and the device recited in claims 1 and 19. Applicants respectfully point out that the specification at page 2, lines 14-15, page 39, lines 5-32 and page 40, lines 28-33 describe the relationship between the pump and valves and the other structural elements of the invention. In addition, applicants have provided Figures 2H and I which show the relationship of the pump and valve to the other elements of the invention. Accordingly, applicants request withdrawal of the rejection.

The Examiner states that claim 18 is indefinite because the applicants fail to recite any element to create fluid flow. Applicants have amended claim 18 and the rejection is moot.

Attached hereto is a marked-up version of the changes made to the claims by the "Restriction and Amendment". The attached page is captioned **"Version with markings to show changes made."**

Please direct any calls in connection with this application to the undersigned at (415) 781-1989.

Dated: 6/15/01

Respectfully submitted,

FLEHR HOHBACH TEST
ALBRITTON & HERBERT LLP

By: Renee M. Kosslak
Renee M. Kosslak, Reg. No. 47,717 for
Robin M. Silva, Reg. No. 38,304

Four Embarcadero Center - Suite 3400
San Francisco, California 94111-4187
Telephone: (415) 781-1989
Facsimile: (415) 398-3249

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"VERSION WITH MARKINGS TO SHOW CHANGES MADE"

In the Specification:

Paragraph beginning at line 4, page 2 has been amended as follows:

Figure 1 depicts some preferred embodiments of the invention. Figure 1A depicts a solid support **5** that has a sample inlet port **10**, a first microchannel **15**, a storage module **25** (for example, for assay reagents) with a second microchannel **20**. The second microchannel (**20B**) may be in fluid contact directly with the detection module **30** comprising a detection electrode **35**, or (**20A**) in contact with the first microchannel **15**. Figure 1B depicts a [sample handling well]cell handling module **40** and a second storage well **25A** with a microchannel **20** to the [sample handling well]cell handling module **40**. For example, the [sample handling well]cell handling module **40** could be a cell lysis [chamber]module and the storage well **25A** could contain lysis reagents. Figure 1C depicts a [sample handling well]cell handling module **40** that is a cell capture or enrichment [chamber]module, with an additional reagent storage well **25B** for elution buffer. Figure 1D depicts the addition of a reaction module **45**, with a storage module **25C**, for example for storage of amplification reagents. Optional waste module **26** is connected to the reaction module **45** via a microchannel **27**. All of these embodiments may additionally comprise valves, waste wells, and pumps, including additional electrodes.

Paragraph beginning at line 9 of page 10 has been amended as follows:

In addition to the flow channel system, the devices of the invention are configured to include one or more of a variety of components, herein referred to as "modules", that will be present on any given device depending on its use. As shown in Figures 2A-2H, [T]these modules include, but are not limited to: sample inlet ports; sample introduction or collection modules; cell handling modules **40** (for example, for cell lysis, cell removal, cell concentration, cell separation or capture, cell growth, etc.); separation modules, for example, for electrophoresis, dielectrophoresis, gel filtration, ion exchange/affinity chromatography (capture and release) etc.; reaction modules **45** for chemical or biological alteration of the sample, including amplification of the target analyte (for example, when the target analyte is nucleic acid, amplification techniques are useful, including, but not limited to polymerase chain reaction (PCR), ligase chain reaction (LCR), strand displacement amplification (SDA), and nucleic acid sequence based amplification (NASBA)), chemical, physical or enzymatic cleavage or alteration of the target analyte, or chemical modification of the target; fluid pumps **120**; fluid valves **130**; thermal modules **110** for heating and cooling; storage modules for assay reagents; mixing chambers; and detection modules.

Paragraph beginning at line 29 of page 10 has been amended as follows:

In a preferred embodiment, the devices of the invention include a cell handling module **40** (Figure 1B and 1C). This is of particular use when the sample comprises cells that either contain the target analyte or that must be removed in order to detect the target analyte. Thus, for example, the detection of particular antibodies in blood can require the removal of the blood cells for efficient analysis, or the cells (and/or nucleus) must be lysed prior to detection. In this context, "cells" include eukaryotic and prokaryotic cells, and viral particles that may require treatment prior to analysis, such as the release of nucleic acid from a viral

particle prior to detection of target sequences. In addition, cell handling modules may also utilize a downstream means for determining the presence or absence of cells. Suitable cell handling modules include, but are not limited to, cell lysis modules **50** (Figure 2A), cell removal modules **60** (Figure 2B), cell concentration modules **70** (Figure 2C), and cell separation or capture modules **80** (Figure 2D). In addition, as for all the modules of the invention, the cell handling module is in fluid communication via a flow channel with at least one other module of the invention.

Paragraph beginning at line 8 of page 11 has been amended as follows:

In a preferred embodiment, the cell handling module **40** includes a cell lysis module **50** (Figure 2A). As is known in the art, cells may be lysed in a variety of ways, depending on the cell type. In one embodiment, as described in EP 0 637 998 B1 and U.S. Patent No. 5,635,358, hereby incorporated by reference, the cell lysis module may comprise cell membrane piercing protrusions that extend from a surface of the cell handling module. As fluid is forced through the device, the cells are ruptured. Similarly, this may be accomplished using sharp edged particles trapped within the cell handling region. Alternatively, the cell lysis module can comprise a region of restricted cross-sectional dimension, which results in cell lysis upon pressure.

Paragraph beginning at line 26 of page 11 has been amended as follows:

In a preferred embodiment, the cell handling module **40** includes a cell separation or capture module **80** (Figure 2D). This embodiment utilizes a cell capture region comprising binding sites capable of reversibly binding a cell surface molecule to enable the selective isolation (or removal) of a particular type of cell from the sample population, for example, white blood cells for the analysis of chromosomal nucleic acid, or subsets of white blood cells. These binding moieties may be immobilized either on the surface of the module or on a particle trapped within the module (i.e. a bead) by physical absorption or by covalent attachment. Suitable binding moieties will depend on the cell type to be isolated or removed, and generally includes antibodies and other binding ligands, such as ligands for cell surface receptors, etc. Thus, a particular cell type may be removed from a sample prior to further handling, or the assay is designed to specifically bind the desired cell type, wash away the non-desirable cell types, followed by either release of the bound cells by the addition of reagents or solvents, physical removal (i.e. higher flow rates or pressures), or even in situ lysis.

Paragraph beginning at line 8 of page 12 has been amended as follows:

In a preferred embodiment, the cell handling module **40** includes a cell removal module **60**(Figure 2B). This may be used when the sample contains cells that are not required in the assay or are undesirable. Generally, cell removal will be done on the basis of size exclusion as for "sieving", above, with channels exiting the cell handling module that are too small for the cells.

Paragraph beginning at line 12 of page 12 has been amended as follows:

In a preferred embodiment, the cell handling module **40** includes a cell concentration module **70** (figure 2C). As will be appreciated by those in the art, this is done using "sieving" methods, for example to concentrate the cells from a large volume of sample fluid prior to

lysis.

Paragraph beginning at line 24 of page 14 has been amended as follows:

In a preferred embodiment, the separation module **80** includes an electrophoresis module **90** (Figure 2E), as is generally described in U.S. Patent Nos. 5,770,029; 5,126,022; 5,631,337; 5,569,364; 5,750,015, and 5,135,627, all of which are hereby incorporated by reference. In electrophoresis, molecules are primarily separated by different electrophoretic mobilities caused by their different molecular size, shape and/or charge. Microcapillary tubes have recently been used for use in microcapillary gel electrophoresis (high performance capillary electrophoresis (HPCE)). One advantage of HPCE is that the heat resulting from the applied electric field is efficiently [disappated]dissipated due to the high surface area, thus allowing fast separation. The electrophoresis module serves to separate sample components by the application of an electric field, with the movement of the sample components being due either to their charge or, depending on the surface chemistry of the microchannel, bulk fluid flow as a result of electroosmotic flow (EOF).

Paragraph beginning at line24 of page 15 has been amended as follows:

In a preferred embodiment, the devices of the invention include a reaction module **45**(Figure 1D). This can include either physical, chemical or biological alteration of one or more sample components. Alternatively, it may include a reaction module wherein the target analyte alters a second moiety that can then be detected; for example, if the target analyte is an enzyme, the reaction chamber may comprise an enzyme substrate that upon modification by the target analyte, can then be detected. In this embodiment, the reaction module may contain the necessary reagents, or they may be stored in a storage module and pumped as outlined herein to the reaction module as needed.

Paragraph beginning at line 24 of page 16 has been amended as follows:

In a preferred embodiment, the reaction module **45** includes a chamber for the biological alteration of all or part of the sample. For example, enzymatic processes including nucleic acid amplification **100** (Figure 2F), hydrolysis of sample components or the hydrolysis of substrates by a target enzyme, the addition or removal of detectable labels, the addition or removal of phosphate groups, etc.

Paragraph beginning at line 16 of page 38 has been amended as follows:

In this and other embodiments, a thermal module **110** (Figure 2G) may be used, that is either part of the reaction chamber **45** or separate but can be brought into spatial proximity to the reaction module. The thermal module **110** can include both heating and/or cooling capability. Suitable thermal modules are described in U.S. Patent Nos. 5,498,392 and 5,587,128, and WO 97/16561, incorporated by reference, and may comprise electrical resistance heaters, pulsed lasers or other sources of electromagnetic energy directed to the reaction chamber. It should also be noted that when heating elements are used, it may be desirable to have the reaction chamber be relatively shallow, to facilitate heat transfer; see U.S. Patent No. 5,587,128.

Paragraph beginning at line1, page 39 has been amended as follows:

In a preferred embodiment, the devices of the invention include at least one fluid

pump **120** (Figure 2H). Pumps generally fall into two categories: "on chip" and "off chip"; that is, the pumps (generally electrode based pumps) can be contained within the device itself, or they can be contained on an apparatus into which the device fits, such that alignment occurs of the required flow channels to allow pumping of fluids.

Paragraph beginning at line 5 of page 39 has been amended as follows:

In a preferred embodiment, the pumps **120** (Figure 2H) are contained on the device itself. These pumps **120** are generally electrode based pumps; that is, the application of electric fields can be used to move both charged particles and bulk solvent, depending on the composition of the sample and of the device. Suitable on chip pumps include, but are not limited to, electroosmotic (EO) pumps and electrohydrodynamic (EHD) pumps; these electrode based pumps have sometimes been referred to in the art as "electrokinetic (EK) pumps". All of these pumps rely on configurations of electrodes placed along a flow channel to result in the pumping of the fluids comprising the sample components. As is described in the art, the configurations for each of these electrode based pumps are [slighly]slightly different; for example, the effectiveness of an EHD pump depends on the spacing between the two electrodes, with the closer together they are, the smaller the voltage required to be applied to effect fluid flow. Alternatively, for EO pumps, the [sampcing]spacing between the electrodes should be larger, with up to one-half the length of the channel in which fluids are being moved, since the electrode are only involved in applying force, and not, as in EHD, in creating charges on which the force will act.

Paragraph beginning at line 28 of page 40 has been amended as follows:

In a preferred embodiment, the devices of the invention include at least one fluid valve **130** (Figure 2H) that can control the flow of fluid into or out of a module of the device, or divert the flow into one or more channels. A variety of valves are known in the art. For example, in one embodiment, the valve may comprise a capillary barrier, as generally described in PCT US97/07880, incorporated by reference. In this embodiment, the channel opens into a larger space designed to favor the formation of an energy minimizing liquid surface such as a meniscus at the opening. Preferably, capillary barriers include a dam that raises the vertical height of the channel [immediated]immediately before the opening into a larger space such a chamber. In addition, as described in U.S. Patent No. 5,858,195, incorporated herein by reference, a type of "virtual valve" can be used.

In the Claims:

Claim 1 has been amended as follows:

1. (Amended) A microfluidic device for the detection of a target analyte in a sample comprising a solid support comprising:

a) a sample inlet port;

b) a [sample handling well] cell handling module comprising a least one well port;

c) a first microchannel to allow fluid contact between said sample inlet port and said [sample handling] well port;

d) a detection module comprising:

i) a detection electrode;

ii) a self-assembled monolayer;

iii) a binding ligand; and
iv) a detection inlet port to receive said sample;
e) a second microchannel to allow fluid contact between said [sample handling] well port and said detection inlet port.

Claim 2 has been cancelled.

Claim 3 has been amended as follows:
3. (Amended) A device according to claim 1 [wherein said sample handling well is a] further comprising a reagent storage well.

Claim 4 has been cancelled.

Claim 5 has been amended as follows:
5. (Amended) A device according to claim [4]1 wherein said cell handling [well]module comprises a cell lysis [well]module.

Claim 6 has been amended as follows:
6. (Amended) A device according to claim [4]1 wherein said cell handling [well]module comprises a cell removal [well]module.

Claim 7 has been amended as follows:
7. (Amended) A device according to claim [4]1 wherein said cell handling [well]module comprises a cell concentration [well]module.

Claim 8 has been cancelled.

Claim 9 has been amended as follows:
9. (Amended) A device according to claim 1 wherein said sample handling [well]module comprises a separation module.

Claim 11 has been amended as follows:
11. (Amended) A device according to claim 1 [wherein said sample handling well comprises]further comprising a reaction module.

Claim 13 has been amended as follows:
13. (Amended) A device according to claim 1 wherein said [sample handling well] reaction module comprises a thermal module.

Claim 18 has been amended as follows:
18. (Amended) A microfluidic device for the detection of a target analyte in a sample comprising a solid support comprising:
a) a sample inlet port;
b) a reagent storage well comprising an outlet port;
c) a detection module comprising:
i) a detection electrode;

ii) a self-assembled monolayer;
iii) a binding ligand; and
iv) a detection inlet port to receive said sample;
d) a first microchannel to allow fluid contact between said sample inlet port and said detection inlet port; [and]
e) a second microchannel to allow fluid contact between said outlet port and said detection module; and
f) a pump.

Claim 19 has been amended as follows:
19. (Amended) A method for the detection of a target analyte in a sample comprising:
a) introducing said sample to a sample inlet port of a microfluidic device comprising a solid support comprising:
i) at least one [sample handling well]cell handling module comprising a well inlet port and a well outlet port;
ii) a first microchannel to allow fluid contact between said sample inlet port and said [sample handling well]cell handling module;
iii) a detection electrode comprising:
1) a self-assembled monolayer;
2) a binding ligand; and
3) a detection inlet port to receive said sample; and
iv) a second microchannel to allow fluid contact between said [sample handling well]cell handling module and said detection inlet port; and
b) detecting the presence of said target analyte.

Claim 20 has been cancelled.

Claim 22 has been cancelled.

Claim 23 has been amended as follows:
23. (Amended) A method according to claim [22]19 wherein said cell handling [well]module comprises a cell lysis [well]module.

Claim 24 has been amended as follows:
24. (Amended) A method according to claim [22]19 wherein said cell handling [well]module comprises a cell removal [well]module.

Claim 25 has been amended as follows:
25. (Amended) A method according to claim [22]19 wherein said cell handling [well]module comprises a cell concentration [well]module.

Claim 26 has been cancelled.

Claim 27 has been amended as follows:
27. (Amended) A method according to claim 19 wherein said sample handling [well]module comprises a separation module.

Claim 28 has been amended as follows:
28. A method according to claim 27 wherein said separation module comprises an electrophoresis module.

Claim 29 has been amended as follows:
29. (Amended) A method according to claim 19 [wherein said sample handling well comprises]further comprising a reaction module.

Claim 31 has been amended as follows:
31. (Amended) A method according to claim 19 wherein said [sample handling well]reaction module comprises a thermal module.